CONTRIBUTION OF ION CHANNEL-BLOCKING BY OXIMES TO THEIR THERAPEUTIC ACTION AGAINST SOMAN POISONING IN VITRO.

John E. H. Tattersall

Biology Division, Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK

ABSTRACT

Oximes, which reactivate inhibited acetylcholinesterase (AChE), are used to treat poisoning by organophosphorus anticholinesterases. Some oximes produce neuromuscular recovery even when the inhibited AChE has undergone dealkylation ("aging") and can no longer be reactivated. It has been proposed that at least part of this "direct" recovery may be due to blockade of nicotinic ion channels. I have tested this hypothesis by comparing the direct recovery produced by a range of these compounds with their channel-blocking activities.

Direct recovery was measured in soman-poisoned diaphragms from guinea pigs. Single channel recordings were made from the endplates of dissociated adult mouse flexor digitorum brevis muscle fibres.

Both oximes and non-oxime analogues could produce direct recovery. The compounds which caused the greatest recovery also produced a very rapid, flickering type of open channel blockade. Several parameters of channel blockade showed strong correlations with the degree of recovery measured in diaphragms over the range of 12 compounds tested. Furthermore, dose-response relationships for direct recovery and channel blockade were very similar. The results support the suggestion that oxime-induced neuromuscular recovery from soman poisoning in vitro is due to ion channel blockade.

COMPONENT PART NOTICE

THIS PAPER IS A COMPONENT PART OF THE FOLLOWING COMPILATION REPORT:

TITLE: Proceedings of the Medical Defense Bioscience Review (1993) in Baltimore, Maryland on 10-13 May 1993. Volume 2.

TO ORDER THE COMPLETE COMPILATION REPORT, USE AD-A275 668.

THE COMPONENT PART IS PROVIDED HERE TO ALLOW USERS ACCESS TO INDIVIDUALLY AUTHORED SECTIONS OF PROCEEDING, ANNALS, SYMPOSIA, ETC. HOWEVER, THE COMPONENT SHOULD BE CONSIDERED WITHIN THE CONTEXT OF THE OVERALL COMPILATION REPORT AND NOT AS A STAND-ALONE TECHNICAL REPORT.

THE FOLLOWING COMPONENT PART NUMBERS COMPRISE THE COMPILATION REPORT:

AD#: AD-P008 795 AD#:

AD#: thru AD#:

AD#: AD-P008 852 AD#:

Accesion For	
NTIS CRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

INTRODUCTION

Oximes are used in the treatment of poisoning by organophosphorus anticholinesterases, and are generally believed to exert their therapeutic effect by reactivating the inhibited acetylcholinesterase (AChE). With some organophosphorus compounds, particularly soman, the inhibited enzyme rapidly undergoes a dealkylation reaction, called aging, to form a complex which cannot be reactivated by oximes (Stares, 1976). Some oximes have been reported to be effective against soman poisoning, even after aging of the inhibited AChE has taken place (Stares, 1976; Hamilton & Lundy, 1989). Furthermore, similar protection against soman has been found with related compounds in which the oxime group required for reactivation is absent (Stares, 1976; Clement, 1981). These observations indicate that such compounds have an additional therapeutic action which is not related to the regeneration of AChE.

The therapeutic effects of some oximes against soman poisoning can be demonstrated in vitro using the guinea pig phrenic nerve-hemidiaphragm preparation (Smith et al., 1981; French et al., 1983). The neuromuscular recovery produced in these experiments is reversed by washing out the oxime and thus appears to be due to a direct pharmacological action. One possible mechanism of this effect is that the oximes may block the ion channel associated with the nicotinic acetylcholine receptor and thus counteract the effects of excessive cholinergic stimulation (Alkondon et al., 1988; Alkondon & Albuquerque, 1989). In order to investigate this possibility, the actions of a range of oximes and related compounds were studied in guinea pig diaphragms in which aging of the soman-inhibited AChE had taken place. The

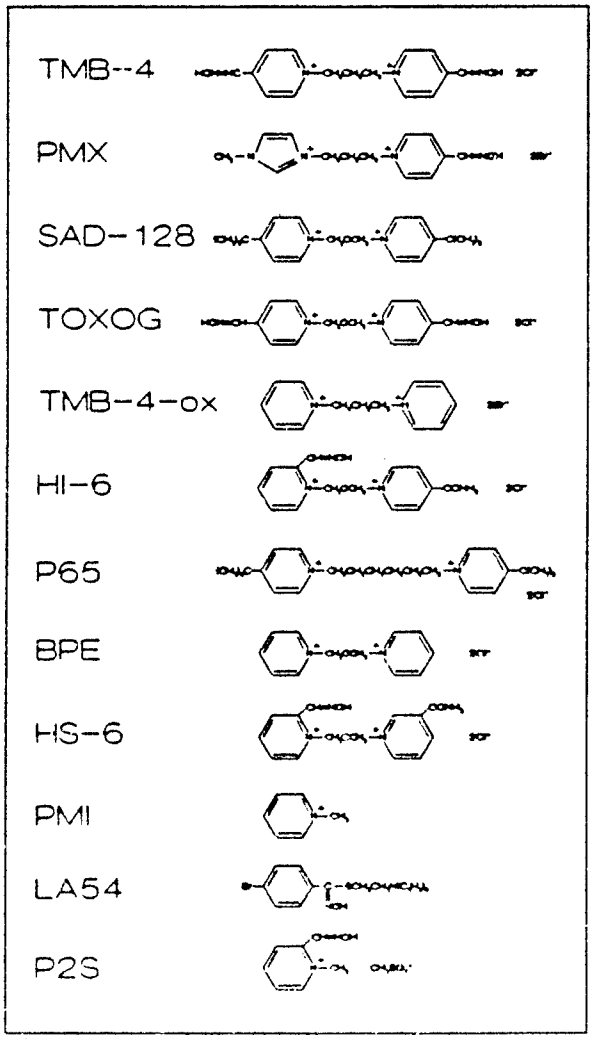

Figure 1. Structures of compounds tested.

concentrations tested were similar to those achieved in blood plasma following therapeutic administration in vivo. These actions were then compared with the effects of the same concentrations of the drugs on nicotinic receptor ion channels in mouse muscle endplates, studied with patch clamp techniques.

METHODS

i) Phrenic nerve-hemidiaphragm experiments

Male Dunkin-Hartley guinea pigs were stunned and killed by exsanguination, and the left and right hemidiaphragms were removed and prepared for recording in a similar manner to that described by Bulbring (1946). They were suspended Tyrode solution and stimulated via the phrenic nerve. The stimulus intensity was adjusted to produce supramaximal stimulation of the preparations. Muscle contractions were recorded from a baseline tension of 4g using an isometric force transducer.

Single twitch responses, elicited every 10s, were recorded throughout the experiment. Every 15min, a 50Hz tetanus was elicited for a duration of 3s. Following control tetani, soman (10^{-7}M) was added to the tissue bath for 30min. The soman was then removed by washing and the preparation was left for 30min to allow the inhibited AChE to age before addition of the test compound. The test compound was added to the bath for 30min. This was then washed off, and 30min later a second dose of soman (10^{-7} M) was added to the tissue bath to inhibit any reactivated AChE. All compounds were tested initially at a concentration of 200μM, this being the highest blood plasma concentration which is usually achieved during studies of oxime therapy in vivo. If a compound significantly blocked twitch contractions at this concentration, it was then tested at lower concentrations. The compounds tested are shown in Figure 1.

Neuromuscular function was assessed by measuring the tetanic tension is into the tetanus. This was expressed as a percentage of the mean of three tetani recorded before the application of soman. Three aspects of neuromuscular recovery following oxime administration were measured as follows: (a) recovery due to direct

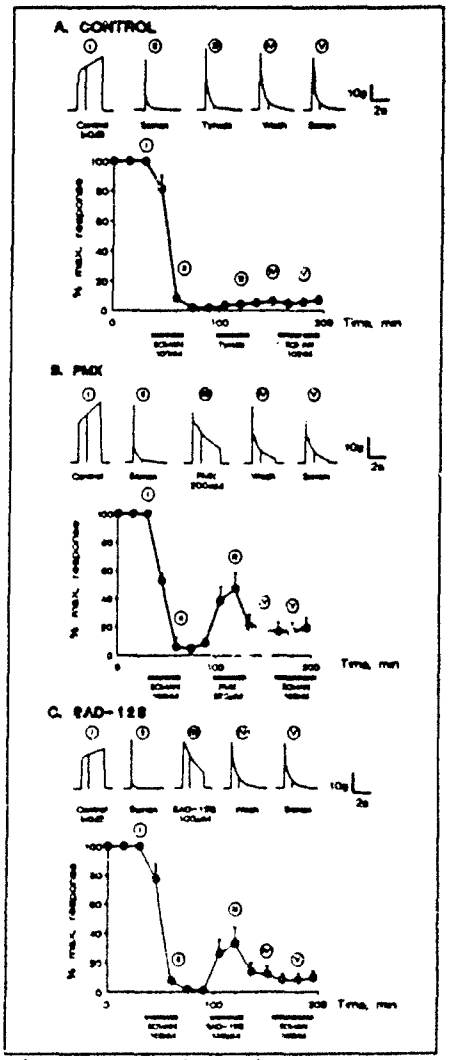

Figure 2. Oximes induce recovery in soman-poisoned diaphragms by a direct action. Graphs show mean ± SE for 4 preparations. Insets are representative contractions at the points indicated.

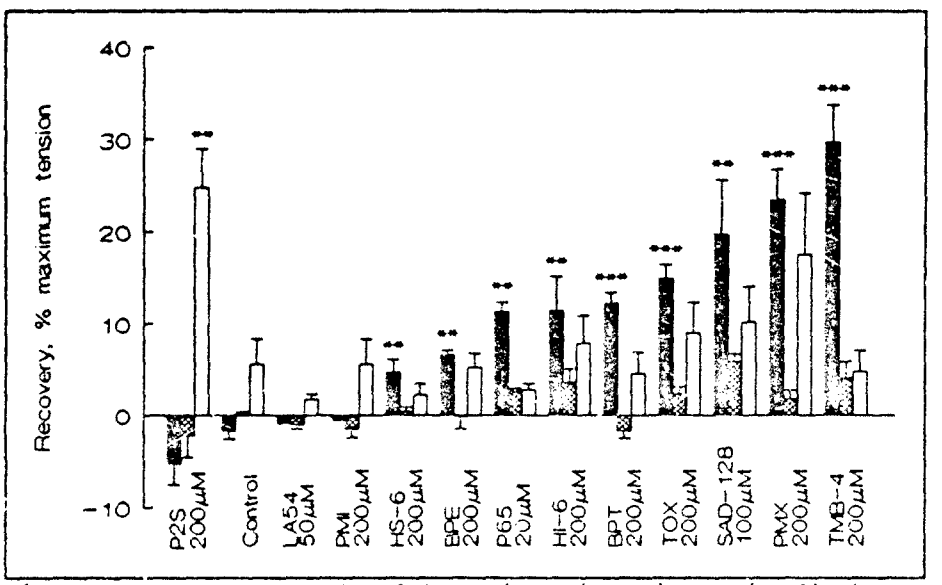

Figure 3. Recovery produced by oximes in guinea pig diaphragm following soman poisoning. Solid columns, direct action; hatched columns, reactivation, open columns, residual recovery. Statistical significances: *P<0.05; **P<0.01; ***P<0.001.

pharmacological actions of the oxime on the muscle was measured by subtracting the response after washing out the oxime from the response before washing; (b) oxime-induced reactivation of acetylcholinesterase was calculated by subtracting the response after reinhibition by the second dose of soman from the response after washing out the oxime but before the second application of soman; (c) the remaining recovery following the second dose of soman was termed residual recovery, since it was due neither to direct action of the oxime nor to reactivation of acetylcholinesterase. The residual recovery has been termed adaptation by Smith et al. (1981).

ii) Single Channel Recordings

The preparation of dissociated adult mouse muscle fibres has been described previously (Tattersall, 1991). Single channel currents were recorded in cell-attached patches at or near the endplate region with an Axopatch-1B amplifier and analysed using the programmes pCLAMP, IPROC and LPPOC (Axon Instruments). Details of the analysis have been described previously (Tattersall, 1993). Most measurements were made at a membrane potential of -80mV, but the conductance was calculated from measurements of single channel amplitudes at a number of different membrane potentials.

RESULTS AND DISCUSSION

Application of soman (100nM) to the diaphragm preparation blocked the sustained phase of the tetanus, so that all that remained was a brief, rapidly decaying contraction (Figure 2). The tension 1s after the start of the tetanus was reduced to less than 5% of its control value by soman. In control experiments, in which no oxime was applied, the blockade was not removed by

washing off the soman, although there was a gradual increase in the tetanic response over time (Figure 2A). This recovery was not due to spontaneous regeneration of active AChE, since it was not blocked by a second application of soman.

A number of the oximes tested, such as PMX, produced a recovery of up to 50% of the tetanic response, by lengthening the decay of the tetanus (Figure 2B). Most of this recovery was reversed by washing off the oxime, and was therefore considered a direct action of the oxime on the muscle. The remaining response was not due to reactivation of AChE, as it was resistant to blockade by a second dose of soman. Similar results were obtained with some of the compounds which lacked the oxime group, such as SAD-128 (Figure 2C).

The results for all 12 compounds tested are summarised in Figure 3. LA54, P65 and SAD-128 significantly blocked muscle twitch at a concentration of 200μM, and so were tested at lower concentrations. None of the drugs caused significant reactivation of AChE, and only one of them, P2S, produced a significant increase in the residual recovery remaining after the second dose of soman. There was, however, a wide variation between compounds in the degree of direct action on the diaphragm. The monopyridinium compounds had no significant direct effect, but many of the bispyridinium drugs and PMX produced a very marked direct recovery of tetanic tension, up to 30% of the control response. These measurements of the direct therapeutic effects of the compounds were subsequently used for comparison with their channel-blocking activities.

Single Channels

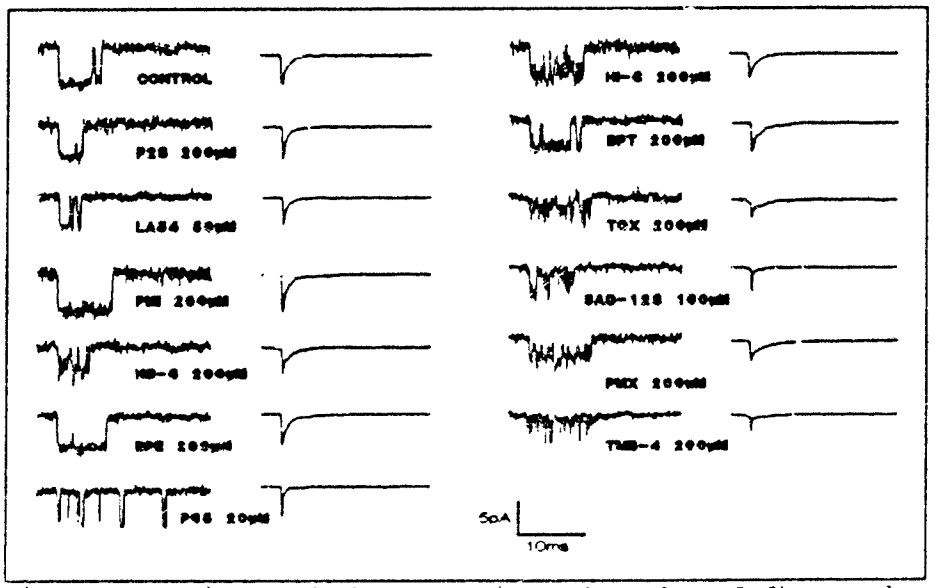

Figure 4. Oximes block open ion channels. Left panels, representative single channel openings. Right panels, averages of 100 openings. The membrane potential was -80mV for each patch.

The drugs were tested on single nicotinic receptor ion channels at the same concentrations which had been used in the diaphragm recovery experiments. Typical recordings are shown in Figure 4. The left hand trace in each panel is a representative channel opening. The right hand trace in each panel shows an ensemble current produced by averaging 100 single channel bursts.

The records are arranged in order of increasing direct pharmacological action measured for each compound in the diaphragm. In control patches and in the presence of drugs which had no significant direct action, the channel openings were simple square wave events interrupted by only a few brief closings, and separated by long silent periods ranging from tens of milliseconds to several seconds. The noise level of the open state remained similar to that during the closed state of the channel. The ensemble currents in these conditions decayed according to a single exponential function.

Compounds which had pronounced direct actions in the diaphragm produced a rapid, flickering block of the channels, so that the openings took the form of bursts of short events. Many of the events were too brief to be completely resolved by the 5kHz bandwidth of the recording equipment, and the apparent amplitude of the single channel current was smaller than in control patches. The noise level of the open state was often greater than that during the closed state, probably reflecting the presence of unresolved brief events. The ensemble currents obtained in the presence of these drugs showed dual exponential decays and smaller peak amplitudes than in control patches.

In order to quantify the channel-blocking properties of the compounds for correlation with their direct actions, several parameters of channel block were measured. It was sometimes difficult to measure these accurately due to the incomplete resolution of individual events, and this gave rise to considerable scatter in the data. Nonetheless, each of these parameters showed a strong correlation with the direct action measured in the diaphragm experiments (Figure 5). Compounds with large direct actions reduced the conductance, mean open time and burst open probability of the channels, and increased the mean burst duration and the mean number of events in a burst.

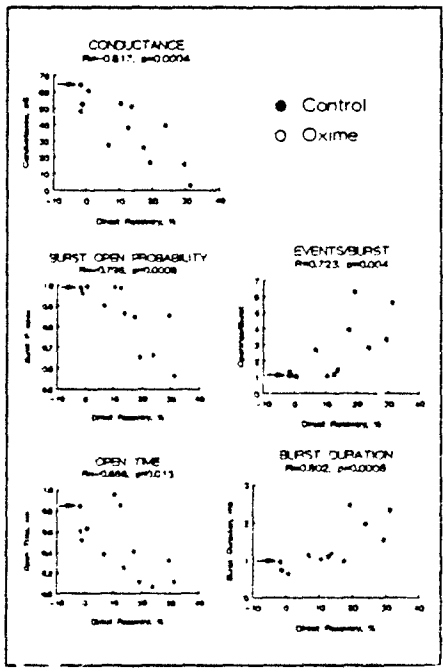

Figure 5. Direct action correlates with open channel blockade. Filled circles, marked by arrows, control values; open circles, values in the presence of oximes or analogues.

Concentration-Response Relationships

If the direct action seen in diaphragms was indeed related to channel blocking by the oximes, the two effects would be expected to show similar concentration dependence. Two of the compounds which showed the greatest effects, PMX and SAD-128, were therefore studied further by investigating the actions of different concentrations on soman-poisoned diaphragms and on single channels. Figure 6 shows concentration-response relationships obtained with PMX. Significant recovery of the diaphragm due to direct action of the oxime

appeared at a concentration of 100μM. In patch clamp experiments, the threshold concentration for effects single channel parameters was very similar, between 50 and 100μM.

With the non-oxime compound, SAD-128, direct recovery appeared at a lower concentration than for PMX, 20μM, but the concentration-response curve was less steep than the one for PMX (Figure 7). Significant effects of SAD-128 on all of the single channel parameters measured appeared at a similar concentration, between 5 and 10μM.

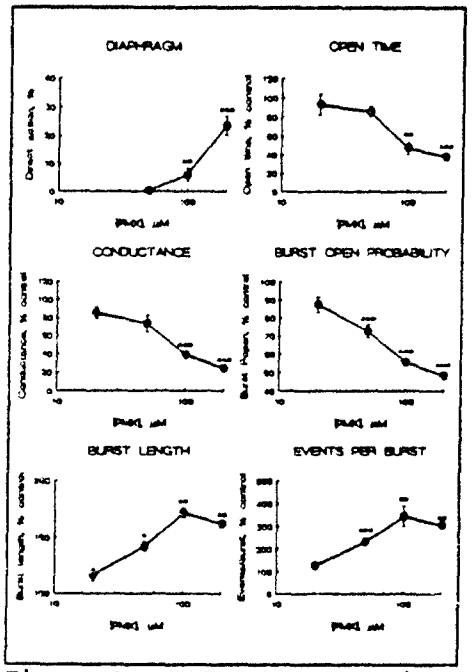

Figure 6. PMX concentration-response relationships. Significant differences from control values: *P<0.05; **P<0.01; ***P<0.001.

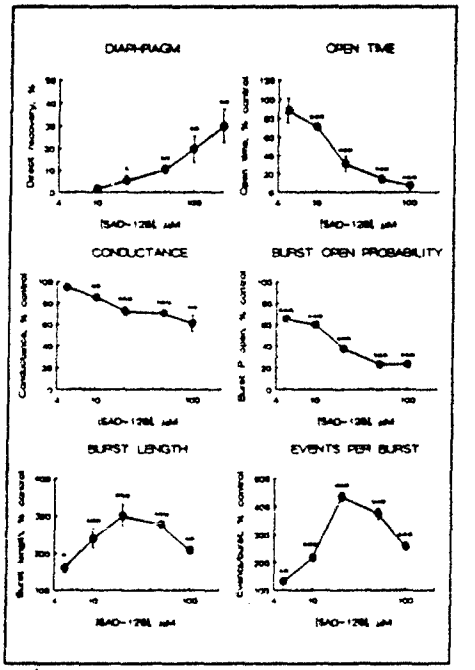

Figure 7. SAD-128 concentration-response relationships. Significant differences from control values: *P<0.05; **P<0.01; ***P<0.001.

Relevance to therapeutic and toxic effects

It is becoming increasingly apparent that mechanisms of action of pyridinium compounds other than reactivation of inhibited AChE can be beneficial in cases of organophosphorus poisoning (Rousseaux & Dua, 1989). Evidence from a number of studies suggests that these alternative mechanisms are more important in the treatment of soman poisoning than against other organophosphates, presumably due to the rapid aging of soman-inhibited AChE (van Helden et al., 1991). It has been suggested that nicotinic receptors are

an important target site (Clement, 1981; Su et al., 1983), and that the channel-blocking action of oximes may be related to their antidotal properties against organophosphates (Alkondon et al., 1988; Alkondon & Albuquerque, 1989).

The present study provides strong evidence that the channel-blocking activity of oximes and analogues does indeed underlie their ability to produce recovery of neuromuscular transmission following soman poisoning in vitro. As pointed out by French et al. (1983), however, these in vitro effects of oximes on neuromuscular function cannot easily be translated to in vivo effects, since the neuromuscular recovery does not correlate well with in vivo protection against soman poisoning. Thus, whilst the channel-blocking activity of oximes appears to underlie their antidotal effects in the in vitro muscle preparation, it cannot fully explain their actions against soman in whole animal protection studies. Other effects may be important, such as ganglion-blocking and antimuscarinic activities (Clement, 1981), and it has been suggested that some of the oximes may cross the blood-brain barrier to have effects on the central nervous system (Rousseaux & Dua, 1989). It is also possible that harmful side effects of some compounds, such as those described for some pyridinium-4-oximes (Schoene, 1976), may diminish their beneficial actions.

Although the therapeutic activity of bispyridinium compounds against soman poisoning is still not fully understood, the results presented here demonstrate how one mechanism of action of these compounds, open channel blocking, could contribute to their overall antidotal effectiveness. Unlike competitive antagonists, which prevent channel activation, channel blockers are able to modulate rather than eliminate nicotinic receptor function. A competitive antagonist, such as tubocurarine, would counteract the effects of ACh by preventing activation of a proportion of nicotinic receptors, but the antagonism could be overcome by increased concentrations of ACh accumulating at the neuromuscular junction and competing with the antagonist. Furthermore, there is a danger that high concentrations of the competitive antagonist would block neuromuscular transmission completely.

In contrast to a competitive antagonist, a channel blocker with rapid kinetics, such as many of the bispyridinium compounds tested here, would reduce the mean current flowing through a channel during a burst without greatly altering the burst length or affecting channel activation. Such an action would provide an effective counter to the overstimulation of nicotinic receptors at an endplate subjected to ACh accumulation following inhibition of AChE. Furthermore, open channel blockade is use-dependent, so that the antagonism would become stronger as more channels were activated. In addition, the rapid cycling of the nicotinic receptor ion channel between blocked and open states could serve as an alternative pathway to protect the receptor from desensitisation (Alkondon & Albuquerque, 1989). A further advantage over competitive antagonists is that high concentrations of such channel blockers would be less likely to block transmission completely. By selecting oximes with optimal channel-blocking properties in addition to their other beneficial activities, it may be possible to improve the effectiveness of current treatments against soman poisoning.

ACKNOWLEDGEMENTS

I am grateful for the generous gifts of toxogonin and HI-6 from Dr. M.G. Hamilton, and of pyrimidoxime from Dr. M.H. Garrigue.

REFERENCES

ALKONDON M., ALBUQUERQUE E.X. (1989). The nonoxime bispyridinium compound SAD-128 alters the kinetic properties of the nicotinic acetylcholine receptor ion

channel - a possible mechanism for antidotal effects. J. Pharmacol. Exp. Ther. 250: 842-852.

ALKONDON M., RAO K.S., ALBUQUERQUE E.X. (1988). Acetylcholinesterase reactivators modify the functional properties of the nicotinic acetylcholine receptor ion channel. J. Pharmacol. Exp. Ther. 245: 543-556.

BULBRING E. (1946). Observations on the isolated phrenic nerve diaphragm preparation. Br. J. Pharmacol. 1: 38-61.

CLEMENT J.G. (1981). Toxicology and pharmacology of bispyridinium oximes - insight into the mechanisms of action vs. soman poisoning in vivo. Fundam. Appl. Toxicol. 1: 193-202.

FRENCH M.C., WETHERELL J.R., WHITE P.D.T. (1983). The reversal by oximes and their deoximinomethyl analogues of neuromuscular block produced by soman. Eur. J. Pharmacol. 91: 399-409.

HAMILTON M.G., LUNDY P.M. (1989). HI-6 therapy of soman and tabun poisoning in primates and rodents. Arch. Toxicol. 63: 144-149.

ROUSSEAUX C.G., DUA A.K. (1989). Pharmacology of HI-6, an H-series oxime. Can. J. Physiol. Pharmacol. 67: 1183-1189.

SCHOENE K. (1976). Kinetic studies on chemical reactions between acetylcholinesterase, toxic organophosphates and pyridinium oximes. In: Medical Protection Against Chemical-Warfare Agents. ed. Stares, J.E.S. pp. 88-100. Stockholm: Almqvist & Wirksell International.

SMITH A.P., VAN DER WIEL H.J., WOLTHUIS O.L. (1981). Analysis of oxime-induced neuromuscular recovery in guinea pig, rat and man following soman poisoning in vitro. Eur. J. Pharmacol. 70: 371-379.

STARES J.E.S. (1976). Medical protection against chemical-warfare agents. In: Medical Protection Against Chemical-Warfare Agents. ed. Stares, J.E.S. pp.157-166. Stockholm: Almqvist & Wirksell International.

SU C.-T., TANG C.P., MA C., SHIH Y.S., LIU C.Y., WU M.T. (1983). Quantitative structure-activity relationships and possible mechanisms of action of bispyridinium oximes as antidotes against pinacolyl methylphosphonofluoridate. Fundam. Appl. Toxicol. 3: 271-277.

TATTERSALL, J.E.H. (1990). Effects of organophosphorus anticholinesterases on nicotinic receptor ion channels at adult mouse muscle endplates. Br. J. Pharmacol. 101: 349-357.

TATTERSALL, J.E.H. (1993). Ion channel blockade by oximes and recovery of diaphragm muscle from soman poisoning in vitro. Br. J. Pharmacol. 108: 1006-1015.